Effects of *Toxoplasma gondii* infection on visual processing

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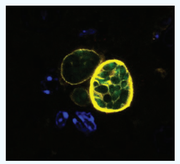
Introduction

Toxoplasma gondii is a single-celled parasite that can establish chronic infection in the brain of its host. Infection abolishes rodents' innate aversion to feline urine odor, increasing the likelihood of the parasite gaining access to its definitive host, the feline. The neural circuit changes underlying this behavior remain mostly undefined. Infection with *T. gondii* has been shown to alter perisomatic inhibitory synapses[1]. These local circuit changes have yet to be reconciled with altered odor processing. A loss of inhibitory synapse function could lead to altered visual response properties. Previous studies have shown widespread enrichment of infiltrating immune cells in neocortex during chronic infection, yet evidence suggests parasite localization is not a fixed phenotype[2]. Altered visual system processing can be studied using cortical responses while an animal views a visual stimulus, looking at cell-type specific responses. Preliminary results suggest *T. gondii* has a transient effect on excitatory and inhibitory cells in bV1. Using this paradigm of studying the effects of *Toxoplamsa* on visual processing can lead to an understanding of how this infection effects other sensory modalities.

Methods

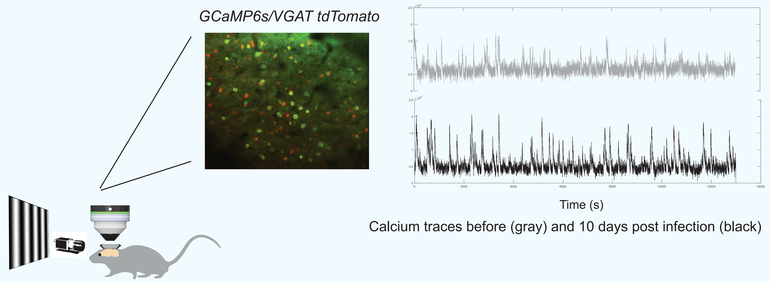
1. Infection with *T. gondii* parasite



T. gondii cysts in the brain. GFP+ T. gondii are found within cysts in the mouse brain. The CST-1 protein (yellow) marks the cysts wall.

Mice were (i.p.) infected with 200 PA7 (type II strain) *T. gondii* tachyzoites. Imaging was done on day 0, day 10, and day 20 post-infection.

2. Measuring single-cell visual response properties in binocular V1 using 2-photon calcium imaging

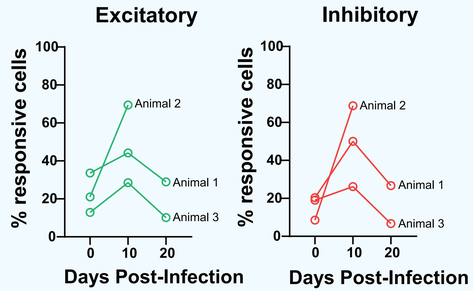


Calcium traces before (gray) and 10 days post infection (black)

Drifting gratings were shown to VGAT-tdTomato+ mice that had been injected with AAV-syn-GCaMP6s 2-3 weeks prior to imaging. Red and green fluorescence were gathered with a resonant two-photon microscope, at a depth of ~200um below the pia. Full field sinusoidal gratings of three spatial frequencies (0.03, 0.06, 0.12) in eight directions (0 –315, 45° steps) at a fixed temporal frequency (2 Hz) were presented, in addition to a blank condition and a condition in which the whole monitor flickered at 2 Hz. Stimulus conditions were presented in a random order for 10 repetitions. For each trial, the stimulus was presented for 2 s, followed by 3 s of gray screen. Viewed through both eyes.

Results

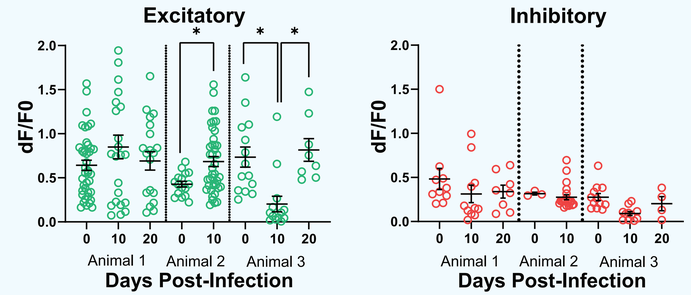
1A. Percent Responsive



Note: Animal 1 died between 10 and 20dpi. Animal 2 died shortly after 20dpi. Animal 3 survived beyond 20dpi.

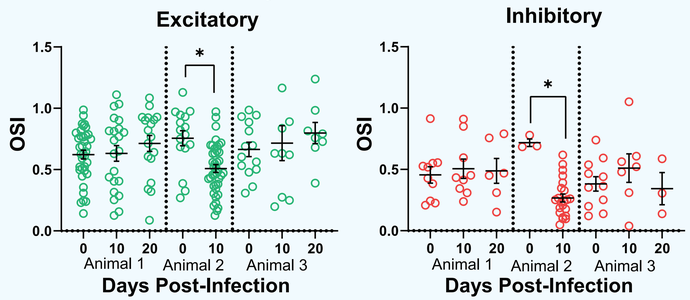
a. Percent of neurons in layer 2/3 bV1 identified as significantly responsive. Percent responsive appears to increase by day 10, then return to near baseline by day 20. (Animal 1, n = 119, 52, 69; Animal 2, n = 76, 59 ; Animal 3, n = 109, 49, 79).

1B. Response Amplitude at Preferred Spatial Frequency



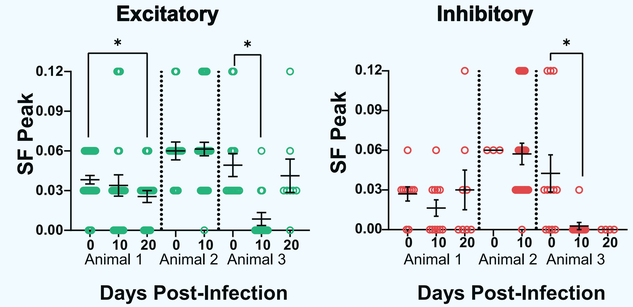
b. Maximum response amplitude measured in layer 2/3 bV1. Changes in excitatory neuron response amplitude vary across animals. Inhibitory neurons show a trend towards a decrease in response size at day 10. (Animal 1, n = 40, 23, 20; Animal 2, n = 16, 41; Animal 3, n = 14, 14, 8). Brown-Forsythe and Welch ANOVA test, Welch's t-test. Error bars represent mean +/- SEM.

1C. Orientation Selectivity



c. Orientation selectivity measured in layer 2/3 bV1. One animal showed a significant decrease in orientation selectivity at 10 days post-infection. The remaining two animals showed a trend towards increasing response size in excitatory neurons. (Animal 1, n = 37, 22, 18; Animal 2, n = 16 , 41; Animal 3, n = 14, 10, 8). Brown-Forsythe and Welch ANOVA test, Welch's t-test. Error bars represent mean +/- SEM.

1D. Spatial Frequency Preference



d. Spatial frequency preference measured in layer 2/3 bV1 before and during *T. gondii* infection. Changes in spatial frequency tuning are inconclusive. (Animal 1, n= 40, 23, 20; Animal 2, n = 16 , 41 ; Animal 3, n = 14, 14, 8). Brown-Forsythe and Welch ANOVA test, Welch's t-test. Error bars represent mean +/- SEM.

Summary

Given the stripping of inhibitory synapses found in other studies, we hypothesized that reduced inhibition would yield broader tuning properties in visual cortex, which is what we found. Initial observations showed a global increase in responsiveness at 10dpi that returned to baseline by 20dpi. A trend toward decreasing response amplitude in inhibitory neurons at 10dpi, that may subside as infection progresses to chronic, was also observed. One animal of three showed a significant shift towards weaker orientation selectivity in both cell types at 10dpi, which was not observed in the other two animals. It should be noted that this particular animal died from the infection much earlier than the others, which may indicate a relation between sickness and effect size. These results suggest that parasite infection affects both inhibitory and excitatory physiology in visual cortex, perhaps differently during acute and chronic infection. Altered sensory processing could impact how a rodent perceives threat, contributing to loss of fear response. Future studies will address whether the parasite consistently contacts this region.

References

1. Carrillo, G.L., et al., Toxoplasma infection induces microglia-neuron contact and the loss of perisomatic inhibitory synapses. *Glia*, 2020. 68(10): p. 1968-1986.

2. Schneider, C.A., et al., Imaging the dynamic recruitment of monocytes to the blood-brain barrier and specific brain regions during Toxoplasma gondii infection. *Proc Natl Acad Sci U S A,* 2019. 116(49): p. 24796-24807.